Thiotepa, busulfan and fludarabine compared to busulfan and cyclophosphamide as conditioning regimen for allogeneic stem cell transplant from matched siblings and unrelated donors for acute myeloid leukemia

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Abstract

Busulfan plus cyclophosphamide (BuCy) is the traditional conditioning regimen for allogeneic stem cell transplant (allo-SCT) for young, fit patients with acute myeloid leukemia (AML). The thiotepa-busulfan-fludarabine (TBF) protocol has recently demonstrated promising outcome in cord blood and haploidentical SCT; however, there is limited evidence about this regimen in transplant from matched siblings (MSD) and unrelated donors (UD). We retrospectively com- pared outcomes of 2523 patients aged 18-50 with AML in remission, undergoing transplant from MSD or UD prepared with either TBF or BuCy conditioning. A 1:3 pair-matched analysis was performed: 146 patients receiving TBF were compared with 438 patients receiving BuCy. Relapse risk was significantly lower in the TBF when compared with BuCy group (HR 0.6, P = .02), while NRM did not differ. No significant difference was observed in LFS and OS between the two regimens. TBF was associated with a trend towards higher risk of grades III-IV aGVHD (HR 1.8, P = .06) and inferior cGVHD (HR 0.7, P = .04) when compared with BuCy. In patients undergoing transplant in first remission, the advantage for TBF in terms of relapse was more evident (HR 0.4, P = .02), leading to a trend for better LFS in favor of TBF (HR 0.7, P = .10), while OS did not differ between the two cohorts. In conclusion, TBF represents a valid myeloablative conditioning regimen providing significantly lower relapse and similar survival when compared with BuCy. Patients in first remission appear to gain the most from this proto- col, as in this subgroup a tendency for better LFS was observed when compared with BuCy.

1 | INTRODUCTION

The concept of conditioning regimen for allo-SCT has been recently reshaped. Historical protocols relied mostly on total body irradiation (TBI), and the combination of cyclophosphamide (Cy) and TBI had been widely used as preparatory regimen for allo-SCT in acute myeloid leukemia (AML) for decades.[1] The introduction of alkylators in substitution of TBI moved the field forward. The combination of busulfan and cyclophosphamide (BuCy) was developed in the late 1980s,[2,3] and since then has remained a standard of care for young, fit patients with AML undergoing allo-SCT. In the last two decades a significant effort has been made to minimize regimen toxicity, leading to the possibility of extending the availability of allo-SCT to elderly and unfit patients.[4,5] This tendency resulted in the development of a plethora of nonmyeloablative (NMA), reduced intensity (RIC) and so-called "reduced toxicity" regimens, in ascending order according to the intensity of myeloablation.[6,7] Nevertheless, recent evidence tempered enthusiasm about the mitigation of conditioning intensity unless necessary (ie, for older and/or frail patients with high comorbidities [HCT-CI] score).[8,9] In fact, standard myeloablative protocols remain today the primary option in young, fit patients with AML, as dose intensity is warranted to control residual disease at transplant while waiting for the graft-vs.-leukemia effect to take over. This concept prompt investigators to develop novel conditioning protocols which could combine an effective anti leukemic activity with an acceptable toxicity profile.

Thiotepa is an alkylating compound which has been included in many preparative regimens for transplant as it holds a good anti tumoral effect in combination with immunosuppressive properties and limited nonhematologic toxicity.[10–12] The combination of thiotepa, busulfan, and fludarabine (TBF) was initially proposed as conditioning regimen for cord blood transplant;[13,14] subsequently, it has been employed for haploidentical transplant demonstrating powerful leukemia control with low relapse rates, thus translating into satisfactory outcome.[15–19] Nevertheless, data reporting the use of TBF pre allo-SCT from matched siblings (MSD) and unrelated donors (UD) are rather limited and mostly preliminary.[20,21] In a recent EBMT study[22] we observed impressive low relapse rate following TBF in a large cohort of AML patients transplanted from MSD or UD. Given the constantly increasing number of allo-SCT performed with the TBF protocol all over Europe, and the lack of a comparison of this regimen with standard myeloablative protocols, we designed the current study to compare outcome of TBF vs. BuCy before allo-SCT from MSD or UD-SCT in young AML patients undergoing transplant in complete remission (CR).

2 | METHODS

2.1 | Study design and data collection

This is a registry-based retrospective study. Data were provided and the study design was approved by the acute leukemia working party (ALWP) of the European society for blood and marrow transplantation (EBMT), in accordance with the EBMT guidelines for retrospective studies. EBMT is a voluntary working group of more than 500 transplant centers which are required to report all consecutive stem cell transplantations and follow-up once a year. Audits are routinely performed to determine the accuracy of the data. Since 1990, patients have been able to provide informed consent that authorizes the use of their transplant information for research purposes. The ALWP of the EBMT granted ethical approval for this study.

We included in the analysis patients with AML aged between 18 and 50 years, who had received either TBF or BuCy as conditioning regimen for MSD or UD SCT in CR between January 2007 and December 2015. All unrelated donors were HLA-matched (10/10) or mismatched at one HLA locus (9/10) by high resolution typing. Patients who received conditioning regimens including oral busulfan, T-depleted grafts, or transplant from <9/10 mismatched unrelated donor were excluded. All patients received myeloablative conditioning regimen (MAC), defined as intravenous (iv) Busulfan dose ≥ 9.6 mg/kg.

2.2 | End-point definitions and statistical analysis

Primary end-points were overall survival (OS) and leukemia-free survival (LFS). Secondary end-points were relapse incidence (RI), nonrelapse mortality (NRM), graft-vs.-host free, relapse-free survival (GRFS), engraftment, incidence and severity of acute (aGVHD) and chronic graft-vs.-host disease (cGVHD). The severity of acute GVHD was graded on an I-IV scale, while cGVHD was scored as mild, moderate or severe in accordance with EBMT standards.[23] LFS was defined

as the interval from transplant to either relapse or death. OS was defined as the time between the date of transplant and the date of death. GVHD free, relapse free survival (GRFS) was defined as survival without the following events: grades 3-4 acute GVHD, severe cGVHD, disease relapse, or death from any cause after transplantation.[24] Probabilities of OS, LFS and GRFS were estimated using Kaplan-Meier curves. Cumulative incidence functions were used to estimate relapse incidence (RI) and nonrelapse mortality (NRM) in a competing risks setting. In order to study acute and chronic GVHD, we considered death and relapse as competing events. A 1:3 pair matched analysis was performed using propensity score matching. Matching factors included age at HSCT, disease status, year of transplant, time from diagnosis to transplant, donor type, CMV serology of donor and recipient, stem cell source, GVHD prophylaxis and use of ATG. The main patient characteristics were compared using Mann-Whitney test for quantitative variables, chi-square test or Fisher exact test for categorical variables. Univariate analyses were performed using the log-rank test for OS, LFS, and GRFS, the Gray test for cumulative incidences. Adjusted hazard ratios comparing TBF vs. BuCy were calculated using the multivariate Cox proportional-hazard model. Proportional-hazard assumption was tested using Schoenfeld residuals. We performed subgroup analysis in patients receiving transplant in CR1 and in TBF patients who received busulfan 9.6 mg/kg when compared with BuCy patients receiving busulfan 12.8 mg/kg. All tests were two sided. The type I error rate was fixed at .05 for statistical testing. 95% Confidence Intervals was provided for time to event outcomes and hazard ratio. Statistical analyses were performed with SPSS 19 (SPSS, Chicago, IL), and R 2.13.2 (R Development Core Team, Vienna, Austria) software packages.

3 | RESULTS

3.1 | Patient, disease and transplant characteristics

Two thousand five hundred and twenty-three patients fulfilled the inclusion criteria for the present analysis; among them, 153 received TBF and 2370 BuCy, respectively. Among all patients, 2044 underwent allo-SCT in first complete remission (CR1) while 479 in subsequent CR. One thousand five hundred and fifty-six patients (62%) were transplanted from MSD, 722 (29%) from 10/10 UD and 245 (9%) from 9/10 UD, respectively. Median follow-up was 22 months. Cytogenetic data were available in 45% of patients; among them, 22% had favorable, 61% intermediate, and 17% adverse cytogenetics.

The TBF group included significantly older patients compared with the BuCy cohort (38 vs. 37 years, $P = .024$). The median year of transplant was 2014 (range 2008-2015) for TBF and 2011 (range 2007-2015) for BuCy, respectively ($P < 10^{-4}$). Patients undergoing TBF were more likely to have received a UD transplant when compared with BuCy (52% vs. 37%, $P < 10^{-4}$). Stem cell source in the TBF group was more frequently BM (35% vs. 25%, $P = .02$) and GVHD prophylaxis CSA + MMF (13% vs. 5%, $P < 10^{-4}$) when compared with BuCy; further, CMV serostatus of donor and patient differed between the two cohorts ($P < 10^{-4}$). Cytogenetic risk, Karnofsky performance score, the proportion of patients who received antithymocyte globulin (ATG) and donor/patient gender match did not differ between the groups. Among the TBF cohort, 64 patients (66%) received busulfan 9.6 mg/kg, while 33 (34%) received 12.8 mg/kg. Within the BuCy cohort, 121 patients (10%) received busulfan 9.6 mg/kg and 1157 (90%) 12.8 mg/kg. Patient characteristics are detailed in Table 1. To reduce inherent bias of a non-randomized comparison between patients receiving the two different regimens, a 1:3 Propensity Score pair-matched analysis was performed, as discussed in the methods section. One hundred and forty-six patients receiving TBF were matched with 438 patients receiving BuCy; the characteristics of the pair-matched populations are detailed in Supporting Information Table S1.

3.2 | Engraftment, nonrelapse mortality and graft-vs.-host disease

Engraftment rate was 100% and 99% following allo-SCT with TBF and BuCy, respectively ($P = .4$). By univariate analysis, nonrelapse mortality was similar between the two cohorts, being 10% and 9% at 1 year, 22% and 13% at 5 years post allo-SCT with TBF and BuCy, respectively ($P = .4$, Figure 1). Leading causes of NRM in the global population were GVHD and infectious complications; the complete list of causes of death and their relative incidence are detailed in Table S2 (Supporting Information).

The incidence of grade II-IV acute graft-vs-host disease (aGVHD) was similar between the two groups, being 29% and 23% in TBF and BuCy, respectively ($P = .2$). However, when restricting the analysis to the incidence of grade III-IV aGVHD, higher rates were observed following TBF (15%) when compared with the BuCy regimen (8%, $P = .04$). By univariate analysis, the cumulative incidence of cGVHD was similar in the two groups, being 36% and 43% at 5 years for TBF and BuCy, respectively ($P = .7$). Similarly, no significant difference was observed in the incidence of severe cGVHD, being 16% in the TBF and 22% in the BuCy group, respectively ($P = .7$). Multivariate analysis confirmed a trend towards higher risk of grade III-IV aGVHD after TBF when compared with BuCy (HR 1.8, CI 1-3.2, $P = .06$). In contrast, a lower risk of cGVHD (HR 0.7, CI 0.5-0.99, $P = .04$) and a trend towards inferior risk of severe cGVHD (HR 0.6, CI 0.3-1.1, $P = .1$) was observed with the TBF regimen when compared with the BuCy protocol.

3.3 | Relapse, leukemia-free survival and overall survival

By univariate analysis, relapse incidence was not statistically different between the two conditioning regimens, being 17% and 21% at 1 year, 25% and 27% at 5 years for TBF and BuCy, respectively ($P = .3$, Figure 1). However, when adjusting for covariates in Cox regression analysis, TBF was associated with significantly lower relapse risk when compared with BuCy (HR 0.6, CI 0.4-0.9, $P = .02$). Leukemia-free survival was not statistically different between the two groups, being 73% and 70% at 1 year, 53 and 50% at 5 years for TBF and BuCy, respectively ($P = .6$). Multivariate analysis confirmed those results (Table 2). Overall survival did not differ as well; it was 80% and 79% at 1 year and 54% and 59% at 5 years for TBF and BuCy, respectively ($P = .9$, Figure 1). These results were confirmed by multivariate analysis.

TABLE 1 Patient, disease and transplant characteristics

	Overall	BuCy	TBF	*P*
Number	2523	2370	153	
Follow-up for survivors (months), median (min-max)	21.8 (1-116.6)	21.7 (1-116.6)	22.4 (1-85.8)	.83
Age of patient at HSCT (years), median (min-max)	37 (18-50)	37 (18–50)	38 (19-50)	.024
Age of patient at HSCT (categorical), *n* (%)				.077
18-39 years old	1531 (60.7)	1449 (61.1)	82 (53.6)	
40-50 years old	992 (39.3)	921 (38.9)	71 (46.4)	
Gender of patient, *n* (%)				.14
Male	1306 (51.8)	1236 (52.2)	70 (45.8)	
Female	1215 (48.2)	1132 (47.8)	83 (54.2)	
Age of patient at diagnosis (in years), median (min-max)	36.5 (14.4-49.6)	36.4 (14.4–49.6)	37.5 (18.3-49.5)	.037
Karnofsky performance at SCT, *n* (%)				.48
10-80	298 (12.9)	282 (13.1)	16 (10.7)	
90-100	2005 (87.1)	1872 (86.9)	133 (89.3)	
Missing	220	216	4	
Cytogenetics, *n* (%)				.36
Good	250 (9.9)	236 (10.0)	14 (9.2)	
Intermediate	696 (27.6)	651 (27.5)	45 (29.4)	
Poor	188 (7.5)	182 (7.7)	6 (3.9)	
Missing	1389 (55.1)	1301 (54.9)	88 (57.5)	
FLT3-ITD, *n* (%)				.26
Absent	296 (60.7)	279 (61.5)	17 (50.0)	
Present	192 (39.3)	175 (38.5)	17 (50.0)	
Missing	2035	1916	119	
Disease stage, *n* (%)				.073
CR1	2044 (81.0)	1930 (81.4)	114 (74.5)	
CR2+	479 (19)	440 (19)	39 (25)	
Year of transplant, median (min-max)	2011.0 (2007.0-2015.0)	2011.0 (2007.0–2015.0)	2014.0 (2008.0-2015.0)	<.0001
Time from diagnosis to SCT (months), median (min-max)	5.4 (0.1-124.2)	5.3 (0.1-124.2)	6.3 (0.3-66.7)	.001
Donor, *n* (%)				<.0001
MSD	1556 (61.7)	1483 (62.6)	73 (47.7)	
UD 10/10	722 (28.6)	670 (28.3)	52 (34.0)	
UD 9/10	245 (9.7)	217 (9.2)	28 (18.3)	
Donor/recipient sex mismatch, *n* (%)				.35
F-M	2020 (81.0)	1892 (80.8)	128 (84.2)	
Other	473 (19.0)	449 (19.2)	24 (15.8)	
Missing	30	29	1	
Stem cell source, *n* (%)				.017
BM	647 (25.6)	594 (25.1)	53 (34.6)	
PBSCs	1852 (73.4)	1752 (73.9)	100 (65.4)	
CMV donor/recipient, *n* (%)				<.0001
−/−	621 (27.3)	605 (28.4)	16 (11.0)	
+/−	211 (9.3)	196 (9.2)	15 (10.3)	
−/+	399 (17.5)	363 (17.1)	36 (24.7)	
+/+	1044 (45.9)	965 (45.3)	79 (54.1)	
Missing				
GVHD prevention, *n* (%)				<.0001
CSA + MTX	2046 (82.7)	1928 (83.0)	118 (77.6)	
CSA + MMF	133 (5.4)	113 (4.9)	20 (13.2)	
CSA	125 (5.1)	113 (4.9)	12 (7.9)	
MTX	88 (3.6)	87 (3.7)	1 (0.7)	
CSA + MMF + MTX	60 (2.4)	60 (2.6)	0 (0.0)	

(*Continues*)

TABLE 1 (Continued)

	Overall	BuCy	TBF	*P*
Other	23 (0.9)	22 (0.9)	1 (0.7)	
ATG used, *n* (%)				.064
No	1770 (70.2)	1652 (69.7)	118 (77.1)	
Yes	753 (29.8)	718 (30.3)	35 (22.9)	
Busulfan dose, *n* (%)				
9.6 mg/kg	185 (13.4)	121 (9.7)	64 (66.2)	
12.8 mg/kg	1190 (86.6)	1157 (90.3)	33 (33.8)	
Other/missing	1148	1092	56	

Data are median (range), *n* (%), or *n*/*N* (%). Some percentages do not add up to 100% because of rounding. Abbreviations: ATG, antithymocyte globulin; BM, bone marrow; BuCy, busulfan-cyclophosphamide; CMV, cytomegalovirus; CR1, first complete remission; CR2+, second or subsequent complete remission; CSA, cyclosporine; FLT3-ITD, fms-*like tyrosine* kinase-3 internal tandem duplication; GVHD, graft vs. host disease; MMF, mofetil mycophenolate; MSD, matched sibling donor; MTX, methotrexate; PBSCs, peripheral blood stem cells; SCT, stem cell transplant; UD, unrelated donor; TBF, thiotepa-busulfan-fludarabine.

The composite endpoint GRFS did not differ between the two study cohorts being 63% and 56% at 1 year, 48% and 40% at 5 years for TBF and BuCy, respectively ($P = .5$).

When examining separately patients receiving allo-SCT from MSD or UD, no significant difference was observed between the TBF and BuCy regimens regarding transplant outcome.

3.4 | Subgroup analysis

We conducted a subgroup analysis in patients receiving transplant in CR1. In this subpopulation, TBF was associated with significantly lower relapse risk when compared with BuCy (HR 0.4, CI 0.2-0.7, $P = .02$), while NRM was not statistically different (TBF vs. BuCy: HR 1.6, CI 0.9-2.8, $P = .15$). Those results led to a trend for better LFS in favor of TBF in this population (HR 0.7, CI 0.5-1.1, $P = .10$), while OS did not differ between the two groups (TBF vs. BuCy: HR 0.9, CI 0.6-1.4, $P = .7$, Figure 2). In this subgroup of patients, the risk of developing aGVHD was not statistically different between the two regimens ($P = .13$), while a lower risk of cGVHD was observed following TBF when compared with BuCy (HR 0.7, CI 0.45-1, $P = .05$), consistently with the results observed in the global population. Notably, GRFS was in favor of TBF with no statistically significant difference (TBF vs. BuCy: HR 0.8, CI 0.5-1.1, $P = .11$).

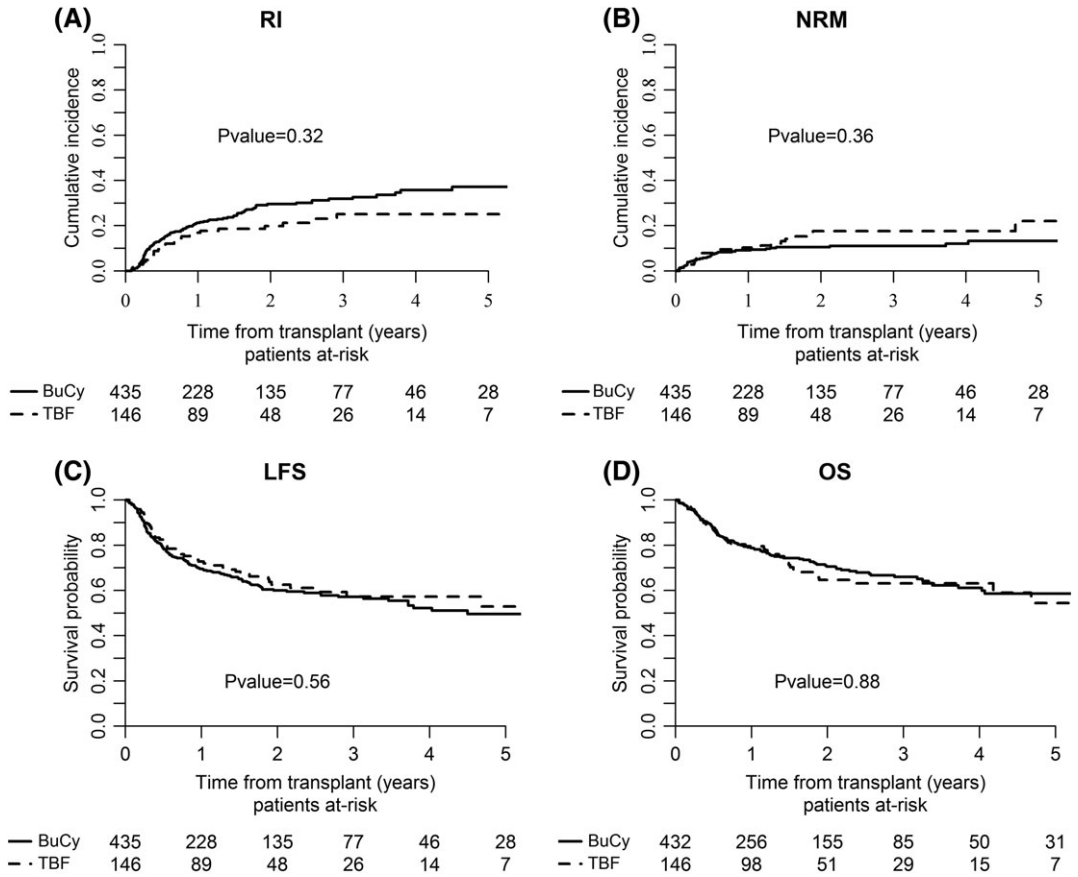

FIGURE 1 Transplant outcome following TBF vs. BuCy conditioning. RI, relapse incidence; NRM, nonrelapse mortality; LFS, leukemia-free survival; OS, overall survival

TABLE 2 Multivariate analysis of transplantation outcome

Conditioning regimen	NRM			RI			LFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
BuCy (reference)	1			1			1			1		
TBF	1.42	(0.84, 2.39)	.19	0.61	(0.40, 0.93)	.02	0.83	(0.60, 1.15)	.27	1.03	(0.73, 1.46)	.87
Conditioning regimen	aGVHD III-IV			cGVHD			Severe cGVHD			GRFS		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
BuCy (reference)	1			1			1			1		
TBF	1.79	(0.99, 3.24)	.06	0.70	(0.49, 0.99)	.04	0.61	(0.34, 1.10)	.10	0.79	(0.59, 1.06)	.12

Abbreviations: BuCy, busulfan-cyclophosphamide; cGVHD, chronic graft-vs.-host disease; GRFS, graft-vs.-host-free, relapse-free survival; LFS, leukemia-free survival; NRM, nonrelapse mortality; OS, overall survival; TBF, thiotepa-busulfan-fludarabine.

Finally, we conducted a subgroup analysis comparing TBF patients who received busulfan 9.6 mg/kg to BuCy patients receiving busulfan 12.8 mg/kg, according to standard regimen schedules. We observed similar RI and NRM after the two regimens with the selected doses of busulfan, leading to similar LFS (BuCy vs. TBF: HR 1.3, CI 0.8-2.3, $P = .28$) and OS (BuCy vs. TBF: HR 1.1, CI 0.6-1.9, $P = .8$). Interestingly, with the reduced dose of busulfan in the TBF regimen, incidence of aGVHD was decreased (cumulative incidence of aGVHD III-IV at 100 day: 10%), this rate being not statistically different when compared with BuCy (BuCy vs. TBF: HR 0.8, CI 0.3-2.1, $P = .7$). In contrast, similarly to what observed in the global population, incidence of cGVHD was inferior following TBF conditioning when compared with BuCy protocol (BuCy vs. TBF: HR 2.4, CI 1.3-4.4, $P = .006$). This result led to a trend for better GRFS for TBF when compared with BuCy in this subgroup of patients (BuCy vs. TBF: HR 1.5, CI 0.96-1.2.5, $P = .07$).

4 | DISCUSSION

The optimal conditioning regimen for young, fit patients with AML is a matter of debate. We hypothesized that the combination of thiotepa, busulfan and fludarabine, which is being increasingly employed all over Europe, could represent a valid myeloablative regimen alternative

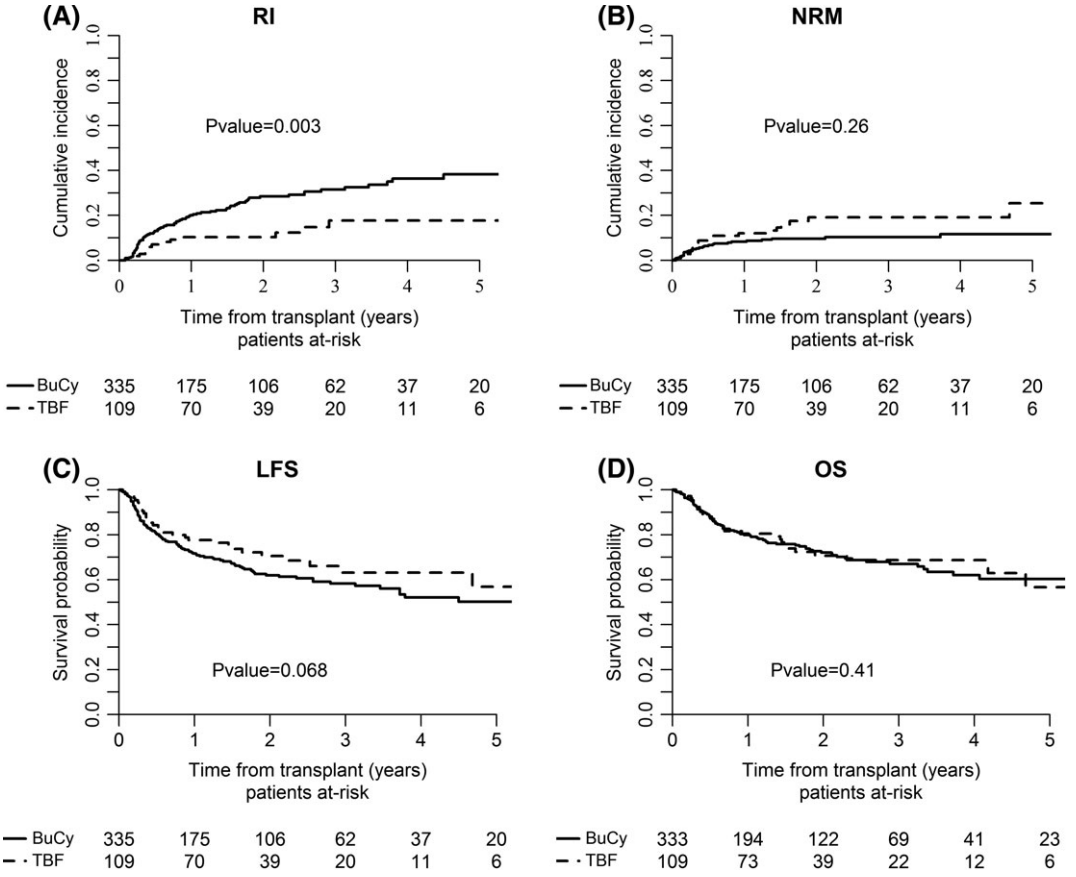

FIGURE 2 Transplant outcome following TBF vs. BuCy conditioning in patients in first complete remission. RI, relapse incidence; NRM, nonrelapse mortality; LFS, leukemia-free survival; OS, overall survival

to BuCy, capable of providing effective leukemia control in allo-SCT from MSD or UD for young patients with AML. In fact, in the present study, we observed significantly lower relapse after the TBF regimen when compared with BuCy, which translated in a trend for better leukemia-free survival in patients transplanted in first remission. Notably, the incidence of relapse was as low as 25% at 5 years following TBF. Moreover, when selecting patients in CR1, relapse was 10% at 1 year and less than 18% at 5 years. When compared with BuCy, the hazard ratio for relapse risk was below 0.5, with high statistical significance. Such results compare favorably with historical data reporting outcome after standard myeloablative regimens; indeed in an EBMT study conducted on a similar population, relapse rate after CyTBI exceeded 20% at 2 years.[25]

In fact, despite optimization of transplant procedures, leukemia recurrence remains the main cause of transplant failure. Further, recent evidence emerging from randomized trials[8,9] demonstrated that mitigating the intensity of the conditioning might not be the right strategy for young, fit AML patients. In accordance to these data, the recent GITMO trial comparing a "reduced toxicity" regimen (BuFlu) with standard BuCy protocol enrolled patients above the age of 40;[26] in this study, the long-term RI did not differ between the two arms and exceeded 35% in the BuCy cohort. In a recent EBMT survey[25] the incidence of relapse was higher following BuCy when compared with CyTBI. Our result of low relapse rate following TBF is consistent with the previous evidence. In the first study reporting this protocol as the preparatory regimen for cord blood transplant,[13] relapse incidence accounted for 18% at 5 years. Subsequently, this conditioning was employed in haplo-SCT by two different Italian groups. In the study led by the centres of Rome and Pescara[18] the use of TBF was the only factor predicting a lower risk of relapse in multivariate analysis. Similarly, Bacigalupo et al.[16] observed a cumulative incidence of relapse related death of 11% for patients transplanted in CR1 following the TBF protocol; this result was recently confirmed by a multicenter trial.[27] In a recent EBMT study,[22] we reported the outcome of TBF regimen in a large cohort of AML patients transplanted from MSD or UD, demonstrating impressive low relapse (14% at 2 years) following this protocol. The latter finding encouraged us to challenge TBF with a standard myeloablative regimen as BuCy, laying the groundwork for the present study. It might be hypothesized that the joint power of two alkylators holding a potent anti-leukemic activity and a significant penetration to sanctuary sites result in effective control of residual disease and prevention of leukemia recurrence.

Importantly, the reduced relapse following TBF translated in a tendency towards better leukemia-free survival in the subgroup of patients transplanted in CR1, this advantage being not evident in the global population. Similarly, no difference was seen in overall survival between the two regimens. Indeed, it should be highlighted that nonrelapse mortality following TBF exceeded 20% at 5 years; this rate was not statistically different when compared with BuCy. This result compares favorably with mortality rates reported in previous studies employing TBF in cord blood (Sanz et al.[13] 5-year NRM: 44%), and haplo-SCT (Arcese et al.[19] cumulative incidence of NRM: 32%). In a recent preliminary report by Sora et al.[28] the authors observed impressively low relapse and nonrelapse mortality rates.

The leading causes of death were represented by disease recurrence, GVHD, and infection. We observed a tendency towards a higher incidence of grades III-IV aGVHD following TBF when compared with BuCy. It might be hypothesized that thiotepa in combination with high dose busulfan is responsible for substantial injury to the gut mucosa, thus leading to the release of pro-inflammatory cytokines triggering aGVHD. In fact, a significant proportion of TBF patients included in this analysis had received busulfan 12.8 mg/kg which, in combination with thiotepa, appears to be excessively toxic, as showed by previous evidence.[22] In fact, when the analysis on TBF was restricted to patients receiving the "standard" schedule with busufan 9.6 mg/kg, the incidence of severe aGVHD was reduced, and resulted similar to the one observed after BuCy. Based on these and previous data,[22] the combination of thiotepa with busulfan 12.8 mg/kg should be avoided, and the "standard" busulfan 9.6 mg/kg schedule should be preferred instead.

Interestingly, we observed lower incidence of cGVHD after TBF when compared with BuCy. This translated into a trend for better GRFS following TBF, which was more evident in the subgroup of patients receiving TBF with busulfan 9.6 mg/kg.

It is important to recognize the limitations of the present study. The retrospective design did not allow to study the reason for patient allocation to a specific regimen, which could have influenced the analysis. Further, some of the patient characteristics differed among the two cohorts; however, this limitation was addressed by performing a propensity score pair-matched analysis based on the most relevant patient, disease and transplant characteristics. Finally, about 50% of the patients lacked informative data about cytogenetic risk. However when we restricted the analysis to patients with available cytogenetics, results were comparable to the global population (data not shown), suggesting a homogeneous distribution of disease risk among the study cohorts. Nevertheless, the retrospective nature of the study does not allow to firmly exclude the theoretic possibility that imbalances between the groups could have influenced at least in part outcome.

In conclusion, our results suggest that TBF represents a valid myeloablative regimen, able to provide significantly lower relapse and similar survival when compared with BuCy. Patients in first remission appear to gain the most from this protocol, as in this subgroup a tendency for better leukemia-free survival was observed when compared with BuCy. Finally, the dose of busulfan within the TBF regimen should not exceed 9.6 mg/kg, as this schedule seems able to retain strong anti-leukemic effect in combination with acceptable nonrelapse mortality. Prospective, randomized trials are warranted to validate our results, aiming at identifying the best myeloablative conditioning regimen for young, fit patients with AML in remission.

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